

DOCKET NO.: NIHA-0194
Application No.: Not Yet Known
Preliminary Amendment - First Action Not Yet Received

PATENT

Amendments to the Specification:

Please insert the following new paragraph on page 1 of the specification following the title:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the National Stage of International Application No. PCT/US2003/033200, filed October 20, 2003, which claims the benefit of U.S. Provisional Application No. 60/420,003, filed October 21, 2002, the disclosure of which is incorporated herein by reference in its entirety.

Please insert the Sequence Listing being filed concurrently herewith into the specification.

Please amend paragraph [0033] on page 8 as follows:

[0001] **FIG 11** shows the CE-ESI-MS results of 100 fmoles of a tryptic digest of horse apomyoglobin. Experimental conditions: As in Fig. 7;

A. Base peak electropherogram;

B. molecular ion scan of the $[M+2H]^{2+}$ ion at $m/z = 690.7$; and

C. CID fragment ion spectrum of the $[M+2H]^{2+}$ ion at $m/z = 690.7$. The CID spectrum was searched against the NCBI non-redundant database using SEQUEST and identified as **HGTVVLTALGGILK** [SEQ ID No: 3] with an Xcorr = 4.95.

Please amend paragraph [0089] on page 25 as follows.

[0002] The separation of a mixture of 9 bioactive peptides at a concentration of 3 $\mu\text{g/mL}$ (injection = 75 fmole) is shown in **FIG. 10**. The inset to this figure shows the corresponding separation of the same bioactive peptide mixture at a concentration of 25 $\mu\text{g/mL}$, using similar CE conditions with UV detection at 214 nm. Compared to UV detection, the relative peak heights vary considerably because of differences in the ionization efficiency of different peptides. Also MS generated peaks are broader, which without being limited to a theory of operation, are due to lack of temperature control in the CE-MS set-up and the slow rate of data acquisition by the MS. The direct detection of MS, however, allowed all of the peptides within this mixture to be easily identified based on their MS spectra. The CE-MS analysis of a tryptic digest of apomyoglobin is shown in **FIG. 11**. In this analysis 100 fmoles of the digest was injected onto the CE column and the peptides were detected in a data-dependent MS/MS mode. The electropherogram for this tryptic digest is shown in **FIG. 11A**. The MS spectrum of the peptide highlighted with an arrow is shown in **FIG. 11B**. The singly and doubly charged species of this peptide were detected with very high S/N, particularly for the doubly charged ion. The tandem MS spectrum of the doubly charged parent ion is shown in **FIG. 11C**. Analysis of this spectrum using SEQUEST identified this peptide as **HGTVVLTALGGILK** [SEQ ID NO: 3] from apomyoglobin with Xcorr score of 4.95. Indeed, an almost complete y ion series was identifiable for this peptide

from the resulting tandem MS spectrum. Table I lists peptides that were identified by tandem MS with high Xcorr. Table II gives a list of all tryptic peptides that were identified by matching the molar mass of ions from the MS spectra with a list of expected peptides generated from the putative protein sequence.

Please amend Table I. on page 27 as follows:

Table I. Identified peptides from CE-MS/MS analysis of horse apomyoglobin tryptic digest.

Peptide	Sequence	Xcorr	Fragment ions
1	HLKTEAEMK (SEQ ID NO: 1)	2.95	14/16
2	YLEFISDAIIHVLHSK (SEQ ID NO: 2)	4.97	22/30
3	HGTVVLTALGGILK (SEQ ID NO: 3)	4.95	23/26
4	YKELGFQG (SEQ ID NO: 4)	2.66	12/14
5	GLSDGEWQQVQLNVWGK (SEQ ID NO: 5)	4.63	21/30

Please amend Table II. on page 28 as follows:

Table II. Identified peptides from CE-MS peptide mapping of horse apomyoglobin tryptic digest.

Fragment	Sequence	Residues
1	GLSDGEWQQVQLNVWGK (SEQ ID NO: 6)	1-16
2	VEADIAGHGQEVLIR (SEQ ID NO: 7)	17-31
3	FDKFK (SEQ ID NO: 8)	43-47
4	HLKTEAEMK (SEQ ID NO: 9)	48-56
5	HGTVVLTALGGILK (SEQ ID NO: 10)	63-76
6	GHHEAELK (SEQ ID NO: 11)	79-86
7	PLAQSHATK (SEQ ID NO: 12)	87-95
8	YLEFISDAIIHVLHSK	102-117

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	<u>(SEQ ID NO: 13)</u>	
9	HPGNFGADAQGAMTK <u>(SEQ ID NO: 14)</u>	118-132
10	ALELFR <u>(SEQ ID NO: 15)</u>	133-138
11	NDIAAK <u>(SEQ ID NO: 16)</u>	139-144
12	YKELGFQG <u>(SEQ ID NO: 17)</u>	145-153